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[Preview Claims](#)[Preview Full Text](#)[Preview Full Image](#)Email Link: **Document ID:** JP 10-045738 A2**ID:****Title:** ANTIBIOTIC SUBSTANCE EPOXYQUINOMICIN C AND D, ITS PRODUCTION AND ANTIRHEUMATIC AGENT**Assignee:** MICROBIAL CHEM RES FOUND**Inventor:**
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ISHIZUKA MASAAKI**US Class:****Int'l Class:** C07D 303/36 A; A61K 31/335 B; C12P 17/02 B; C12P 17/02 J; C12R 01/01 J**Issue Date:** 02/17/1998**Filing Date:** 07/29/1996**Abstract:**

PROBLEM TO BE SOLVED: To obtain a new compound having a new molecular skeleton and exhibiting antirheumatic activity.

SOLUTION: The antibiotic substances epoxyquinomicin C and D are expressed by the formula (R is H for epoxyquinomicin C and C1 for epoxyquinomicin D). The epoxyquinomicin C has the following physical and chemical properties; appearance and nature, white powder having weakly acidic nature; melting point, 168-172°C (decomposition); specific rotation, $[\alpha]D25=+128^\circ$ (c=1.0, methanol); etc. The compound of the formula can be produced by culturing a microbial strain capable of producing epoxyquinomicin C and D such as Amycolatopsis sp. MK299-95F4 in a nutrient medium at

pH6. 5-7.5 under aerobic condition.

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(表1) コラーゲン誘発肉芽抑制作用

被検化合物	投与量 (mg/kg/日)	一回中の マウス数	スコア	
			5回目	6回目
对照	-	8	9.25±1.35	9.00±1.44
エボキシキノマイシンA	2	8	2.00±1.03*	3.63±0.70**
	4	5	2.00±0.84**	1.20±0.58**
エボキシキノマイシンB	1	5	3.00±1.34*	3.00±1.34*
	2	5	2.25±0.85**	3.50±1.71*
エボキシキノマイシンC	2	5	6.40±0.87	6.80±0.97
	4	5	1.60±0.51**	2.40±0.98**

スコア：平均値±標準誤差
対照群との間の有意性 *p < 0.05, **p < 0.01

【0015】エボキシキノマイシンAの2mg/kg、4mg/kg、エボキシキノマイシンBの1mg/kg、2mg/kg、およびエボキシキノマイシンCの4mg/kgは有意に抑制作用を示す通りである。この抗腫瘍スベクタルは日本化学会法会議準拠法に準拠し、ミュラーーピントン基天培地で数希少のスコアを抑制した。

【0016】B) 抗活性

本実験による抗生物質エボキシキノマイシンCおよびD

(表2)

試験薬	最低発育阻止濃度 (μg/ml)	
	エボキシキノマイシンC	エボキシキノマイシンD
スタヒロコッカス・アラクレス・スミス	50	>50
スタヒロコッカス・アラクレス・スミス IS 8610	100	100
スタヒロコッカス・アラクレス IS 10326	100	100
バストレ・ビンダ SP 6395	50	50

【0018】C) 培養細胞培養抑制活性
各種の培養細胞を用いて培養細胞の増殖を30%抑制するエボキシキノマイシンCおよびDの濃度

度 (IC₅₀ 値) を、MTT法 (Journal of Immunological Methods) 65巻、55-60頁 (1983) 参照) で測定した。

その結果を表3に示す。

【0019】

(表3)

供試薬	IC ₅₀ (μg/ml)	
	エボキシキノマイシンC	エボキシキノマイシンD
マウス白血病 L1210	>100	>100
マウス IMCカカルノーマ	>100	>100
エールリヒ	>100	>100
マウス黑色腫 B16-BL6	>100	>100

【0020】D) 常性
IC系活性マウスにエボキシキノマイシンCおよびエボキシキノマイシンDの10mg/kgを腹腔内静脈投与した。まが死亡個体はなく、また毒性症状も見られなかった。また、エボキシキノマイシンCの1mg/kg/日を1週間に3回、合計6週間腹腔内に投与したが死亡個体および毒性症状を示す個体は見られなかった。エボキシキノマイシンCの恒常動物における毒性は非常に低い。

【0021】表2の結果から明らかのように、本発明によるエボキシキノマイシンCおよびDは、特定の細胞に対して弱い抗腫瘍活性を有するから抗腫瘍として有用である。しかし、表2の結果から明らかのように、エボキシノマイシンCおよびDは各種の癌細胞の増殖を100μg/mlで抑制しなかった。

【0022】さらに第2の本発明によれば、アミコラトブシス属に属する、前記の一般式(1)のエボキシキノマイシンCおよびDの生産菌を培養培地に培養し、その後植物からエボキシキノマイシンDを採取することを特許とする、抗生物質エボキシキノマイシンCおよびD(または)エボキシキノマイシンDの製造方法を提供される。

【0023】第2のが発明の方法で使用できるエボキシキノマイシンCおよびDの生産菌は、アミコラトブシス sp. MK299-35F4株がある。この菌株は平成6年10月、微生物学研究所において、宮城県仙台市の土壤より分離されが放線菌で、MK299-35F4の菌株番号が付された微生物である。

【0024】この収量299-95F4株の菌学的性状を次に記載する。

1. 形態
基質系はよく分枝し、シクダグ状を呈する。また分枝が認められる。気管系は直状あるいは不規則な曲状で、円筒形～壺円形の断片または胞子嚢の構造に分分析する。
2. 形態
(7) イースト・愛媛県天培地 (27°C培養)
うす黄茶 (21g, Mustard Tan) の発育上に、白の菌糸をうつすらと生じ、溶解性色素は認められない。
3. 形態
(8) イースト・愛媛県天培地 (27°C培養)
うす黄茶 (31c, Lt. Wheat) の発育上に、白の菌糸をうつすらと生じ、溶解性色素は認められない。
4. 形態
(9) イースト・愛媛県天培地 (27°C培養)
うす黄茶 (21g, Lt. Wheat) の発育上に、白の菌糸をうつすらと生じ、溶解性色素は認められない。
5. 形態
(10) イースト・愛媛県天培地 (27°C培養)
うす黄茶 (21g, Lt. Wheat) の発育上に、白の菌糸をうつすらと生じ、溶解性色素は認められない。
6. 形態
(11) イースト・愛媛県天培地 (27°C培養)
うす黄茶 (21g, Lt. Wheat) の発育上に、白の菌糸をうつすらと生じ、溶解性色素は認められない。
7. 形態
(12) イースト・愛媛県天培地 (27°C培養)
うす黄茶 (21g, Lt. Wheat) の発育上に、白の菌糸をうつすらと生じ、溶解性色素は認められない。

(8) オートミール寒天培地 (ISP-培地3、27°C培

〔0030〕ところで、W₂₉₉-954株の固形成分は、細胞壁にメソ型の2-デジアミノペリドリン酸、アラビノース及びガラクトースを含み、細胞壁タイプIV型を示した。固形体内の過酸化水素を含むノース、ガラクトースを含むA型であった。グリコレートテストの結果はアセル型であった。また、ミコール酸は含有せず、リン脂質はP11型（ホスファチジルエタノールアミン）を含みホスファチジルコリン及び未知のグルコサミン含有リン脂質を含まない、主要なメチオニンはMK-9 (H₄) であった。脂肪酸は16:0, 1-15:0, 16:1, 1-16:0及び17:0を主成分とした。

〔0031〕以上の結果よりみて、W₂₉₉-954株はアミコラトアシス(haemolyticus) 属（文献：「International Journal of Systematic Bacteriology」 36卷, 29-37頁, 1986年）に属するものと考へられる。アミコラトアシス属の既知菌種を検索すると、アミコラトアシス・フルフレア(haemolyticus sulphureus)（文献1）：同上, および文献2、「International Journal of Systematic Bacteriology」 36卷, 29-37頁, 1986年）に記載される。

〔0027〕3. 生理性質

〔0028〕(1) 生育温度範囲

〔0029〕グルコース・アスパラギン基天培地（グルコース1.0%、レーアスパラギン0.05%、リノ酸水素ニカリウム0.05%、じひ天1.0%、pH7.0）を用い、10°C, 20°C, 24°C, 27°C, 30°C, 37°Cおよび50°Cの各温度で試験した。結果、27°C, 30°C, 37°Cでの生育は認められず、20°C～37°Cまでの範囲で生育した。生育至適温度は27°C附近と思われる。

は、その形態上、基生葉系はよく分枝し、シグザク状を呈する。気葉系は直状あるいは不規則な曲線を呈し、分岐を認めない。葉片形は断片または胞子葉の構造に分類される。輪生葉、単葉系、胞子葉のう及び運動性胞子は認められない。種々の場所で、無色～うす黄～黄茶の経験的上に白の氣葉系を生着する。一部の場所で管状葉色葉は前あるいは茶を併げる。メラニン性色素の生成、スルターチの水解性及び活性物質の週回反応がいずれも陰性で 50

合は特に制約されるものではなく、広範囲に亘って使える
ことができ、使用するエギギシノマイシンおよびD
生産菌によって、脳膜の炭酸ガスの組成が配合割合は、
当事者であれば随分小規模実験により容易に決定する
ことができる。また、上記の炭酸ガス割合からなる発酵地
は、培養に先立ち殺菌することができ、この殺菌の前ま
では後で、培地のpHは6~8が適切。特にpH 6.5~
7.5の範囲に調節するのが有利である。

【003.5】 カルバム酸菌のエギギシノマイシン
CおよびD生産菌の培養は、一般の放線菌による抗生物
質の製造において通常使用されている方法に準じて行な
うことができる。通常は好気条件下に培養するのが好適
であり、攪拌しながら及び/または通風しながら行なう
ことができる。また、培養方法としては静置培養、振とう

う培養、透析操作などもないう液内培養のいわゆるものも使用可能であるが、液内培養がエポキシノマイシンCおよびDの大量生産に適している。
【0036】使用する培養温度はエポキシノマイシンCおよびD生産菌の発育適温的に明確されず、通常生物質を生産する範囲であれば、特に制限されるものではなく、使用する生産菌に応じて選択できるが、特に好ましいのは25-30°Cの培養槽内の温度を擧げることができる。培養は通常はエポキシノマイシンCおよびDが十分に蓄積するまで供給することができる。その培養時間は培地の組成や培養温度、使用菌種、使用生産菌株などにより異なるが、通常は72-120時間の培養で目的の抗生物質を得ることができる。培養中の培地内のエポキシノマイシンCおよびDの蓄積量はスピヒコロッカス・アラレクサン・ミスを使用して、通常の抗生物質の定めに用いられる円筒平板法により定めることができる。
【0037】かくして培養物中に蓄積されたエポキシノマイシンCおよびDは、これを培養物から採取する。培養後、必要により、滅菌、過濾分離などのそれ自体公知の分離方法によって培養物から菌体を除した後に、その培養密度を数値 (pH 2-4) に調整し有機溶媒、特に酢酸エチルなどを用いたクロマトグラフ由や、吸着やイオン交換能を有したクロマトグラフィー、ゲルろ過、向流分配を利用したクロマトグラフィーを単独または、組み合わせて使用することにより単離精製する。まくは各種のイオン交換樹脂を用いることができる。また、分離した菌体からは、適当な有機溶媒を用いた溶解抽出法や酵素液による溶出法により菌体から目的の抗生物質を抽出し、上記と同様に単離精製することができ る。かくして、前記した特性を有する新規化合物エポキシノマイシンCおよびDが得られる。
【0038】さらに、第3の本明細では、前述の一例式

(1)

(1)

(式中、RはエボキシキノマイシンCでは水素原子を示す、またエボキシキノマイシンDでは塩素原子を示す)で表わされる化合物である抗生物質エボキシキノマイシンCおよびエボキシキノマイシンD、ならびに次の二式

(8)

13

合は特に制約されるものではなく、広範囲に亘って使える
ことができ、使用するエポキシキノマイシンCおよびD
生産菌によって、量過の供給源の組成が配合割合は、
当事者であれば随分簡単に決定することができる。
たゞ、上記の供給源からなる培养地は、
は、培養に先立ち殺菌することができる、この殺菌の前ま
たは後で、培地のpHを6-8の範囲、特にpH 6.5-
7.5の範囲に調節するのが有利である。

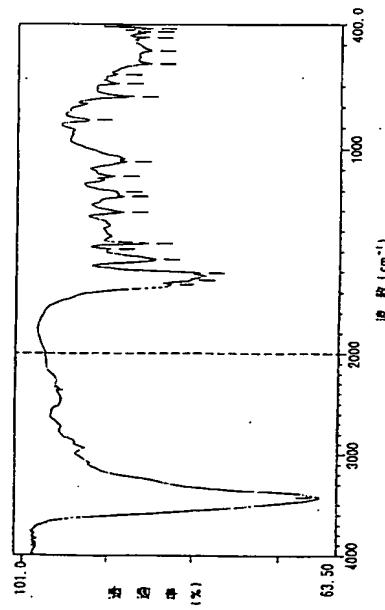
【003.5】かかる供給物のエポキシキノマイシン
CおよびD生産菌の培養は、一般の放線菌による抗生物
質の製造において通常使用されている方法に準じて行な
うことができる。通常は好気条件下に培養するのが好適
であり、攪拌しながら及び/または通風しながら行なう
ことができる。また、培養方法としては静置培養、振とう
培養、通風振とうなどもうちう液内培養のいずれも使用可
能であるが、液内培養がエポキシキノマイシンCおよび
Dの大量生産に適している。

【003.6】使用しうる培養温度はエポキシキノマイシン
CおよびD生産菌の異種間に依存するが、通常
生物質を生産する温度であれば、特に明記されず、酰抗
菌特に好ましいのは25-30°Cの培養内の温度を擧げること
ができる。培養は通常はエポキシキノマイシンCおよび
Dが十分に蓄積するまで供給することができる。その培
養時間は培地の組成や培養温度、使用菌度
株などにより異なるが、通常は72-120時間の培養で目
的の抗生物質を得ることができる。培養中の培地内のエ
ポキシキノマイシンCおよびDの蓄積量はスピロコッ
カス・アラレクサ・スミスを使用して、通常の抗生物質
の定量化用いられる円筒平板法により定量化することがで
きる。

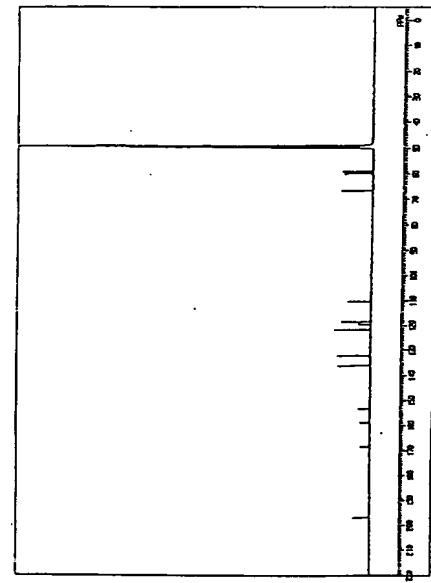
【003.7】かくして培養物中に蓄積されたエポキシキ
ノマイシンCおよびDは、これを培養物から採取する。
培養後、必要により、滅菌、過塩分処理などのそれ自体公
知の分離方法によって培養物から菌体を除した後に、
その培養液を酸性化(pH 2-4) に調整し有機溶媒、特
に酢酸エチルなどを用いた有機溶媒抽出や、吸着イオン交
換法を利用したクロマトグラフィー、ゲルろ過、向流分
配を利用してクロマトグラフィーを単純または、組み
合わせて使用するにより単離精製して目的の抗生物
質を採取することができる。吸着イオン交換法を有す
るクロマトグラフィー用担柱としては、性能が、シリカ
ゲル、多孔性シリスチレンジビニルベンゼン樹脂もし
くは各種のイオン交換樹脂を用いることができる。ま
た、分離した菌体からは、適当な有機溶媒を用いた溶媒
抽出法や酵母細胞による溶出法により菌体から目的の抗
生物質を抽出し、上記と同様に単離精製することができ
る。かくして、前記した特性を有する新規化合物エポキ
シノマイシンCおよびDが得られる。

【003.8】さもなくば、第3の本明細では、前記の一殷式

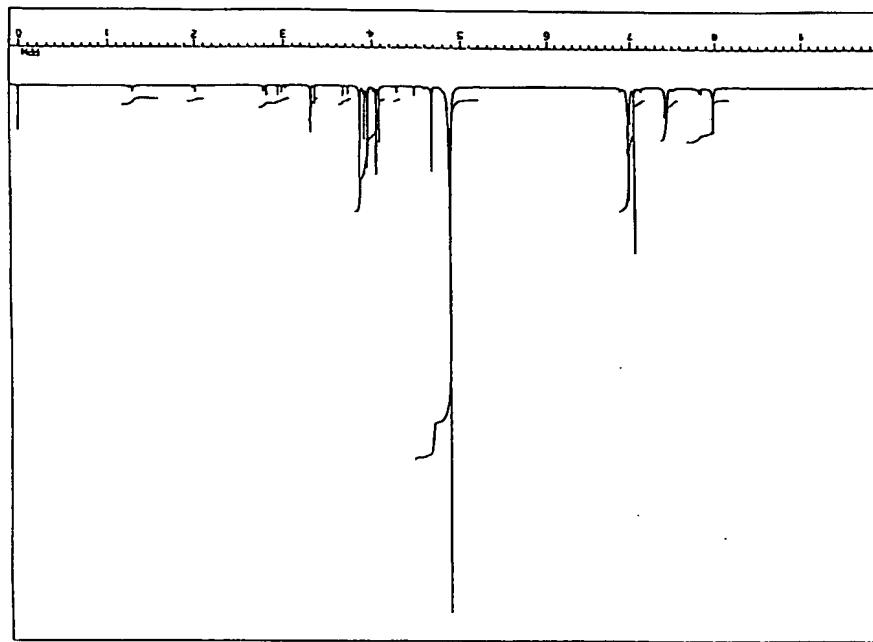
【図2】



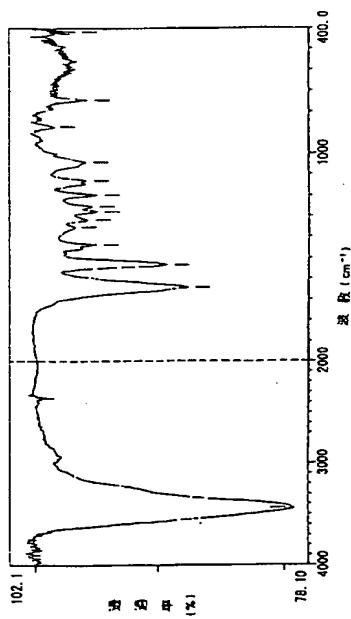
【図3】



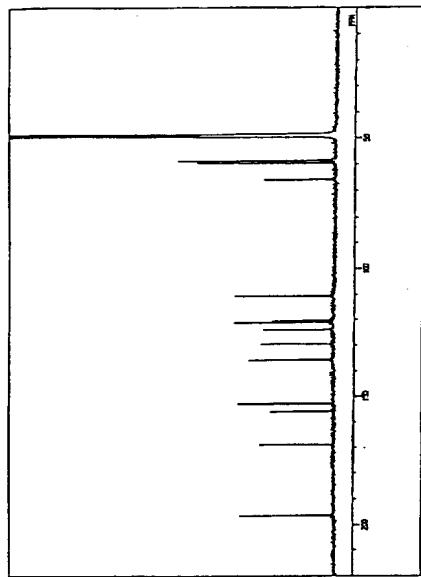
【図4】



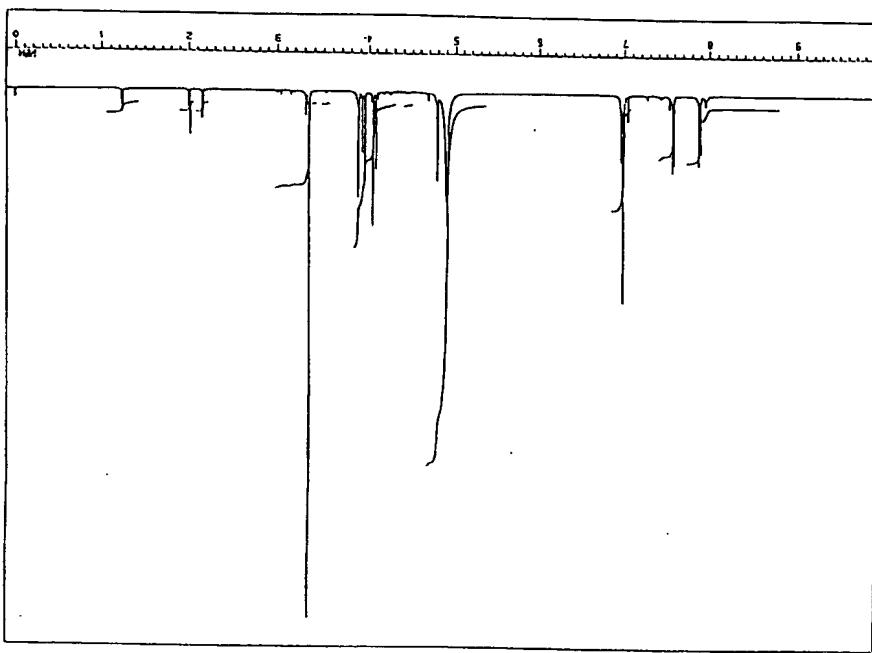
【図6】



【図7】



【図8】



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PATENT ABSTRACTS OF JAPAN

(11) Publication number : 10-045738

(43) Date of publication of application : 17.02.1998

(51) Int.Cl.

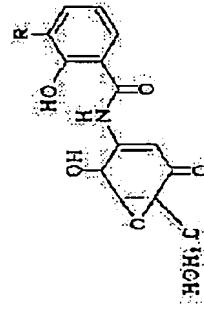
C07D303/36
A61K 31/335
C12P 17/02
//C12P 17/02
C12R 1-01)

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ISHIZUKA MASAAKI(54) ANTIBIOTIC SUBSTANCE EPOXYQUINOMICIN C AND D, ITS PRODUCTION AND
ANTIRHEUMATIC AGENT

(57) Abstract:

PROBLEM TO BE SOLVED: To obtain a new compound having a new molecular skeleton and exhibiting antirheumatic activity.

SOLUTION: The antibiotic substances epoxyquinomicin C and D are expressed by the formula (R is H for epoxyquinomicin C and Cl for epoxyquinomicin D). The epoxyquinomicin C has the following physical and chemical properties: appearance and nature, white powder having weakly acidic nature; melting point, 168-172° C (decomposition); specific rotation, [α]_{D25=+128°} (c=1.0, methanol); etc. The compound of the formula can be produced by culturing a microbial strain capable of producing epoxyquinomicin C and D such as *Amycolatopsis* sp. MK299-95F4 in a nutrient medium at pH 6.5-7.5 under aerobic condition.

LEGAL STATUS

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application converted registration]
[Date of final disposal for application]

[Patent number]

[Date of registration]

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* NOTICES *

quinomycin A and showing a hydrogen atom by epoxy ring mycin B among a formula), and which

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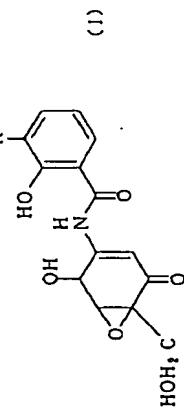
2.*** shows the word which can not be translated.

3. In the drawings, any words are not translated.

CLAIMS

[Claim(s)]

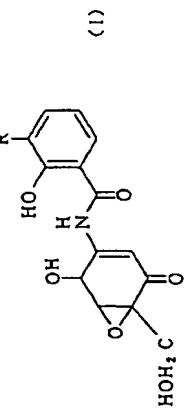
[Claim 1] The following general formula (1)



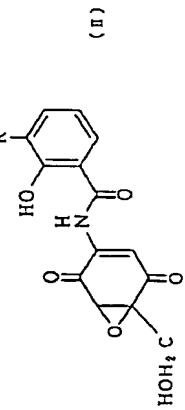
They are the antibiotic epoxy **kino mycin C**, which is expressed with (R showing a hydrogen atom by **kino mycin C**, and showing a chlorine atom by **epoxy kino mycin D** among a formula which is a compound and **epoxy kino mycin D** or those salts).

and which is a compelling and especially killing kind of art; or

[Claim 3] The following general formula (1)



They are the antibiotic epoxy *kino mycin C* which is expressed with (R showing a hydrogen atom by epoxy *kino mycin C*, and showing a chlorine atom by epoxy *kino mycin D* among a formula) and which is a compound *enoyloxy mycin D* and the following general formula (II)



It is the rheumatism agent characterized by containing at least one compound chosen from the antibiotic epoxy quinomycin A which is expressed with (R showing a chlorine atom by epoxy

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] this invention relates to the manufacturing method of epoxy kino mycin C and (or) epoxy kino mycin D, concerning the epoxy kino mycin (Epoxyquinomicin) C which is the new molecular entity in which anti-rheumatism activity is shown and epoxy kino mycin D, or these salts, furthermore, this invention relates to the antirheumatic which makes an active principle epoxy kino mycin C and (or) epoxy kino mycin D, epoxy quinomycin A and epoxy kino mycin B, or at least one compound in those salts.

[0002]

[Description of the Prior Art] The antibacterial substance of various large number is known, and the anticancer matter of various large number is known. On the other hand, the steroid, the acid anti-inflammatory agent, or the immunity modifier is used for the conventional rheumatism therapy.

[0003]

[Problem(s) to be Solved by the Invention] In the chemotherapy of the microbism, to carry out the discovery or the invention of a new compound whose known antibacterial compound which is known conventionally or is used shows the antimicrobial activity which has the different chemical structure and was excellent is always desired. Moreover, the anticancer matter is always wanted to discover or invent the anticancer matter with which there is much what generally has strong toxicity, and toxicity has the low and new chemical structure, and research for it is done.

[0004] moreover, it is a problem that there are various side effects in the steroid and immunity modifier which were used under the conventional rheumatism therapy, and an acid anti-inflammatory agent is symptomatic therapy --- etc. --- an appearance of a very effective antirheumatic is desired from the problem. Then, or, it is effective in the therapy or prevention of rheumatism and there is no side effect, it is requested that a weak new antirheumatic is offered. One of the main purposes of this invention is to offer a new antirheumatic.

[0005]

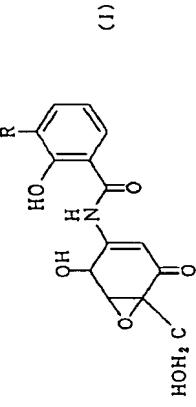
[Means for Solving the Problem] Previously, this invention persons have promoted development of an antibiotic more useful than before and research of utilization for the purpose of offering a new antibiotic with antimicrobial activity and antitumor activity. Consequently, the Amycolatopsis sp. MK 299-55F4 share which succeeded in separating the strain which belongs to the Amycolatopsis group as a new microorganism from a soil sample, and was named about this strain found out producing two or more antibiotics which have a new soil skeleton. It succeeded in isolating two sorts of these new antibiotics, and each was named epoxy quinomycin A and epoxy kino mycin B. Furthermore, it found out having the antitumor activity which shows antimicrobial activity to the gram-positive bacteria with which these new antibiotics contain drug resistance bacteria (methicillin resistant bacteria etc.), and controls growth of a cancer cell (refer to the Japanese Patent Application-No. 7 No. -315542 specification of the December, Heisei 7 four-day application).

[0006] Furthermore, it found out that the aforementioned epoxy quinomycin A and aforementioned B production bacillus which belong to the Amycolatopsis group although this

invention persons advanced research consequently produced two sorts of new compounds of another, although a chemical structure frame is common in epoxy quinomycin A and B. It succeeded in isolating two sorts of these new compounds this time, and each was named epoxy kino mycin C and epoxy kino mycin D.

[0007] Moreover, since this invention persons were doing wholeheartedly research which searches the matter in which anti-rheumatism activity is shown out of the metabolite of a microorganism, they studied whether the epoxy kino mycin C discovered this time and epoxy kino mycin D would have anti-rheumatism activity. Consequently, the epoxy kino mycin C in connection with this invention and epoxy kino mycin D found out controlling the collagen induction arthritis which is the animal experiment model of rheumatoid arthritis. Moreover, it found out that the epoxy quinomycin A and the epoxy kino mycin B which this invention persons discovered previously had anti-rheumatism activity. This invention was completed based on these knowledge.

[0008] In addition, although the epoxy kino mycin C which this invention persons newly got this time, and epoxy kino mycin D showed weak antimicrobial activity to specific bacteria, it was admitted that the activity which controls growth of various kinds of cancer cells was quite low. [0009] Therefore, it sets to the 1st this invention and is the following general formula. (1)



The epoxy kino mycin C which is expressed with (R showing a hydrogen atom by epoxy kino mycin C, and showing a chlorine atom by epoxy kino mycin D among a formula) and which is a compound and epoxy kino mycin D, or these salts are offered.

[0010] Epoxy kino mycin C and D is the weak acidic matter, and has a salt with organic bases, such as quaternary ammonium salt, or a salt with various metals, for example, a salt with alkali metal like sodium, as those salts, and these salts also have the above-mentioned anti-rheumatism activity.

[0011] next, antibiotic epoxy kino mycin C and D is physicochemical -- description is indicated. (1) epoxy kino mycin C is physicochemical -- description -- A appearance and property: -- white fine particles and weak acidic matter B melting point: 168 to 172 degree C (decomposition)

C) specific-rotation: -- Rf value: of [alpha] D 25+128" (c 1.0, methanol) DTLC -- as an expansion solvent with the thin-layer chromatography of 0.31 silica gel (Art. 105715, Merck Co. make) When it developed and measures with a chloroform-methanol (10:1) E mass spectrum (m/z) : 292(M+H) + 290(M+H) - F high-resolution mass spectrum: Experimental value 292.0821(M+H)+ Calculated value 292.0804G molecular formula: -- C14H13NO6H ultraviolet absorption spectrum: -- a continuous line shows UV absorption spectrum measured in (i) methanol solution to drawing 1 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon) (i) 297 (17430) 0.01Ns A dotted line shows UV absorption spectrum measured in the NaOH-methanol solution to drawing 1 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon) A broken line shows UV absorption spectrum measured in 304 (18270) and a 364 (9750) (ii) 0.01N HCl-methanol solution to drawing 1 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon) 296(18140) infrared absorption spectrum (KBr briquette method): It is shown in drawing 2 of an accompanying drawing.

numax (cm⁻¹) 3431, 1604, 1537, 1460, 1309, 1232, 1065, a 750J 13 C-NMR spectrum (CD3 OD/TMS); It is shown in drawing 3 of an accompanying drawing.

K) $^1\text{H-NMR}$ spectrum (CD3 OD/TMS) : it is shown in drawing 4 of an accompanying drawing.
 (2) epoxy kino mycin D is physicochemical — description — A appearance and property: — yellowish brown fine particles and weak acidic matter B melting point: 163 to 168 degree C (decomposition)

C) specific-rotation: — Rf value: of [alpha] D 25+142°(c 1.0, methanol) DTLC — as an expansion solvent with the thin-layer chromatography of 0.10 silica gel (Art.105715, Merck Co. make) When it developed and measures with a chloroform-methanol (10:1) E mass spectrum (m/z) : 326(M+H) + 324(M+H) - F high-resolution mass spectrum: Experimental value 326.0431(M+H)+ Calculated value 326.0417G molecular formula: — C14H12NOG CH ultraviolet absorption spectrum: — a continuous line shows UV absorption spectrum measured in (i) methanol solution to drawing 5 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon) (ii)299 (17590) 0.01Ns A dotted line shows the absorption spectrum measured in the NaOH-methanol solution to drawing 5 of an accompanying drawing. The main peaks are as follows.

A broken line shows UV spectrum measured in lambdamax nm (epsilon)304 (18950) and a 367 (9230) (iii) 0.01N HCl-methanol solution to drawing 5 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon)297(18530) 1 infrared absorption spectrum (KBr briquette method): It is shown in drawing 6 of an accompanying drawing.

numax (cm⁻¹) 3438, 1643, 1533, 1281, a 1200J 13 C-NMR spectrum (CD3 OD/TMS): It is shown in drawing 7 of an accompanying drawing.

K) $^1\text{H-NMR}$ spectrum (CD3 OD/TMS) : it is shown in drawing 8 of an accompanying drawing, furthermore, antibiotic epoxy kino mycin C and D is biological — description is indicated below.

[0012] A) The effectiveness over collagen induction arthritis depressant action collagen induction arthritis was investigated using DBA / 1J male mouse of one groups 5-8. That is, the type II collagen was emulsified with the complete adjuvant of Freund of the amount of isochore,

and 1mg [Vml] administration liquid was produced. It is in the hide of the ridge section of a mouse about this. 0.1ml was inoculated and sensitization was carried out. Type II collagen emulsified on the operating instructions same after three weeks. Intraperitoneal [of a mouse]

was medicated with 0.1ml, the booster was performed, and arthritis was made to induce.

[0013] 2 mg/kg of epoxy kino mycin A and C of epoxy kino mycin or 4 mg/kg and 1 mg/kg of epoxy kino mycin B, or 2 mg/kg were injected intraperitoneally a total of six weeks 3 times from the day of the first collagen inoculation at one week. The depressor effect of collagen induction arthritis was evaluated with the score (the apex of the sum total of four legs is 16) of 0-4 by extent of the rubor of a forelimb and a hind foot, swelling, and a tetany. When a symptom is not seen at all, as for a score 0, facets, such as a finger of the limbs, one score 1 The rubor, When swelling is shown, comparatively big joints, such as 2 or more or a wrist, and an ankle, a score 2 The rubor, [a facet] When swelling is shown, the case where a score 3 judges that the score 4 reached the maximum further and the overall swelling of one hand or guide peg is moreover accompanied by the tetany of a joint when one hand and the whole guide peg show the rubor and swelling is shown, respectively. A result is shown in Table 1.

[0014]

(#1) コラーゲン誘導関節炎抑制作用

試験化合物	投与量 (mg/kg/日)	一回中の マウス数		スコア
		5頭目	6頭目	
エポキシカス・アラレックス	—	8	9.25±1.25	9.00±1.44
エポキシカス・マイシンA	2	6	2.00±1.03*	3.83±0.70**
エポキシカス・マイシンB	4	5	2.00±0.84**	1.20±0.56**

スコア : 平均±標準誤差
対照群との間の有意差 * p < 0.05. ** p < 0.01

[0015] 2 mg/kg of epoxy quinomycin A, 4 mg/kg, 1 mg/kg of epoxy kino mycin B, 2 mg/kg, and 4 mg/kg of epoxy kino mycin C controlled the score of arthritis intentionally.
 [0016] B) The minimum growth inhibition concentration to the various bacteria of the antibiotic epoxy kino mycin C and D by antimicrobial activity this invention is as being shown in the next table 2. This antimicrobial spectrum was measured with the multiple dilution method by ***** and the Mueller HINTON agar medium by the Japanese Society of Chemotherapy standard method.

[0017]

試験菌	最低発育阻止濃度 (μg/ml)	
	エポキシカス・マイシンC	エポキシカス・マイシンD
スタヒロコカクス・アラレックス	50	>50
スタヒロコカクス・アラレックス MS 9510	100	100
スタヒロコカクス・アラレックス MS 10536	100	100
バストラ・ビシシダ sp 6395	50	50

[0018] C) The concentration (IC50 value) of the epoxy kino mycin C which controls growth of a

cancer cell 50% using the cancer cell of cancer cell growth control activity various kinds, and epoxy kino mycin D was measured by the MTT method ("Journal of Immunological Methods" refer to 65 volumes, and 55 - 60 pages (1983)). The result is shown in Table 3. [0119]

供試細胞種	I C ₅₀ (μg/ml)
エンドキノコ エンドキノコ マッシュ	>100
マッシュ L1210 マッシュ 1 MCカナルン - ラ エ - ル ラ ラ マッシュ B16 - BL6	>100 >100 >100 >100
	>100 >100 >100 >100

[0020] D) Although intraperitoneal single-dose administration of 100 mg/kg of epoxy kino mycin C and epoxy kino mycin D was carried out to the toxic ICR system male mouse, there is no death individual and a toxic symptom was not seen, either. Moreover, although it medicated intraperitoneal one with 4 mg/kg / day of epoxy kino mycin C 3 times and a total of six weeks at one week, the individual which shows a death individual and a toxic symptom was not seen. The toxicity over the homeotherm of epoxy kino mycin C is very low.

[0021] Since the epoxy kino mycin C and D by this invention has weak antimicrobial activity to specific bacteria, it is useful as an antimicrobial agent, so that clearly from the result of Table 2. However, epoxy kino mycin C and D is growth of various kinds of cancer cells so that clearly from the result of Table 3. It did not control by ml in 100microg /.

[0022] furthermore, according to the 2nd of this invention, the production bacillus of the epoxy kino mycin C and D of the aforementioned general formula (1) belonging to the Amycolatopsis group is cultivated to a nutrition culture medium, and the manufacturing method of the antibiotic epoxy kino mycin C characterized by extracting epoxy kino mycin C and (or) epoxy kino mycin D from the culture and (or) epoxy kino mycin D is offered.

[0023] As an example of the production bacillus of the epoxy kino mycin C and D which can be used by the approach of the 2nd of this invention, it is Amycolatopsis. sp. MK299-95F4 There is a stock, in a microorganism national chemical laboratory, this strain is the Actinomycetes separated from the soil of Sendai, Miyagi, and will be the microorganism to which the strain number of MK299-95F4 was given in October, Heisei 6.

[0024] This MK299-95F4 share mycology-description is indicated below.

1. Branch gestalt radical viable cell yarn well, and it presents the letter of zigzag. Moreover, fragmentation is accepted. Aerial mycelia have the shape of direct, and the shape of irregular music, and are divided in the fragment of a cylindrical shape - an ellipse, or the spore's structure. The front face is smooth and magnitude is abbreviation. It is 0.4 to 0.6x1.1-1.6 microns, a whorl branch, ******, and a spore obtain and a movement sexual spore is not accepted.

[0025] 2. The color harmony manual (color harmony manual of Container Corporation of America) of the container corporation OBU United States was used for the criterion shown in [] about the publication of the growth condition color in various culture media.

(1) Sucrose and a nitrate agar medium (27-degree-C culture)

On colorless growth, white aerial mycelium is grown slightly, and soluble coloring matter is not accepted.

(2) Glucose asparagine agar medium (27-degree-C culture)

Growing white aerial mycelium on growth of light yellow [Zea, Lt Wheat-2g, Bamboo], soluble coloring matter wears yellow.

(3) Glyceral asparagine agar medium (5 or 27 degrees-C culture of ISP-culture media)

Growing white aerial mycelium on growth of light yellow tea [3ie, Camel -3le, Cinnamon], soluble coloring matter wears yellow-brown.

(4) Starch and a mineral salt agar medium (4 or 27 degrees-C culture of ISP-culture media)

On colorless growth, white aerial mycelium is grown slightly, and soluble coloring matter is not accepted.

(5) Thycosin agar medium (7 or 27 degrees-C culture of ISP-culture media)

Growing white aerial mycelium on growth of light yellow tea [2ig, Mustard Tan] - gray, tint yellow-brown [3ig, Adobe Brown], soluble coloring matter presents light yellow tea.

(6) Nutrient agar medium (27-degree-C culture)

White aerial mycelium is slightly grown on growth of light yellow [Zea, Lt Wheat], and soluble coloring matter is not accepted.

(7) Yeast and a malt-agar culture medium (2 or 27 degrees-C culture of ISP-culture media)

White aerial mycelium is slightly grown on growth of light yellow tea [3ic, Lt Amber], and soluble coloring matter is not accepted.

(8) Oatmeal agar medium (3 or 27 degrees-C culture of ISP-culture media)

White aerial mycelium is slightly grown on growth of colorlessness - light yellow [1 1/2ca, Cream], and soluble coloring matter is not accepted.

(9) Starch agar medium (27-degree-C culture)

On colorless growth, white aerial mycelium is grown slightly, and soluble coloring matter is not accepted.

(10) Malic-acid lime agar medium (27-degree-C culture)

On colorless growth, white aerial mycelium is grown slightly, and soluble coloring matter is not accepted.

[0027] 3. Physiological property (1) As a result of examining using a growth temperature requirement glucose asparagine agar medium (glucose 1.0% and L-asparagine 0.05%, potassium phosphate 0.05%, string agar 3.0%, pH7.0) at each temperature of 10 degrees C, 20 degrees C, 24 degrees C, 27 degrees C, 30 degrees C, 37 degrees C, and 50 degrees C, growth at 10 degrees C and 50 degrees C was not accepted, but be grown in 20 degrees C - 37 degrees C. Growth optimum temperature is considered to be near 27 degree C.

(2) Hydrolysis of starch (starch and a mineral salt agar medium, the ISP-culture medium 4 and a starch agar medium, and all are cultivated 27 degrees C)

In the culture for 21 days, it is negative also in which culture medium.

(3) Generation of melanin Mr. coloring matter (tryption yeast broth, ISP-culture medium 1:peptone yeast and an iron agar medium, an ISP-culture medium 6; thymosin agar medium, the ISP-culture medium 7; all are cultivated 27 degrees C)

Also in which culture medium, it is negative.

[0028] (4) Availability of a carbon source (9; 27 degrees-C culture of PLURIDOHAMU GODORIBU agar-medium and ISP-culture media)

It grows using D-glucose, D-fructose, an inositol, and D-mannitol, and L-arabinose, sucrose, rhamnose, and a raffinose are not used. It is not [the existence of use of D-xylose] ascertained.

(5) The dissolution of malic-acid lime (a malic-acid lime agar medium, 27-degree-C culture)

The dissolution of malic-acid lime is accepted around [after culture] the 10th, and the operation is whenever [middle].

(6) The reduction reaction of a nitrate (8 or 27 degrees-C culture of 0.1% potassium-nitrate content peptone water and ISP-culture media)

It is negative.

[0029] If the above description is summarized, on the gestalt, MK299-95F4 share will branch radical viable cell yarn well, will present the shape of JIGUZAKU, and will accept fragmentation. Aerial mycelia have the shape of direct, and the shape of irregular music, and are divided in the fragment of a cylindrical shape - an ellipse, or the spore's structure, a whorl branch, ******, and a spore obtain and a movement sexual spore is not accepted.

Growing white aerial mycelium on growth of light yellow [Zea, Lt Wheat-2g, Bamboo], soluble coloring matter wears yellow.

(3) Glyceral asparagine agar medium (5 or 27 degrees-C culture of ISP-culture media)

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Also in which culture medium, it is negative.

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It grows using D-glucose, D-fructose, an inositol, and D-mannitol, and L-arabinose, sucrose, rhamnose, and a raffinose are not used. It is not [the existence of use of D-xylose] ascertained.

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The dissolution of malic-acid lime is accepted around [after culture] the 10th, and the operation is whenever [middle].

(6) The reduction reaction of a nitrate (8 or 27 degrees-C culture of 0.1% potassium-nitrate content peptone water and ISP-culture media)

It is negative.

[0029] If the above description is summarized, on the gestalt, MK299-95F4 share will branch radical viable cell yarn well, will present the shape of JIGUZAKU, and will accept fragmentation. Aerial mycelia have the shape of direct, and the shape of irregular music, and are divided in the fragment of a cylindrical shape - an ellipse, or the spore's structure, a whorl branch, ******, and a spore obtain and a movement sexual spore is not accepted.

(2) Glucose asparagine agar medium (27-degree-C culture)

<http://www4.ipdl.ncipi.go.jp/cgi-bin/tran.web.cgi/eje>

a spore obtain and a movement sexual spore is not accepted. By various culture media, white aerial mycelium is grown on growth of colorlessness light yellow - light yellow tea. Soluble coloring matter wears yellow or yellow-brown by a part of culture media. Each of generation of melanin Mr. coloring matter, water solubility of starch, and reduction reactions of a nitrate is negative.

[0030] By the way, the MK299-95F4 share fungus body component showed the cell wall type IV mold to the cell wall including the 2,6-diaminopimelic acid, the arabinose, and the galactose of a meso mold. The reducing sugar in [all] a fungus body were A molds containing arabinose and a galactose. The result of a glycocalyx test was an acetyl mold. Moreover, mycolic acid was not contained, but phospholipid was a PII mold (phosphatidylcholine and strange glucosamine content phospholipid are not included including phosphatidylethanolamine), and main menaquinones were MK-9 (H4), a fatty acid — 16:0, i-15:0, 16:1, and i-16:0 and 17:0 were used as the principal component.

[0031] Seeing from the above result, MK299-95F4 share is Amycolatopsis (Amycolatopsis). Group (reference: "International Journal of Systematic Bacteriology" 36 volumes, 29 - 37 pages, 1986). It is thought that it belongs. Retrieval of the known strain of the Amycolatopsis group raised Amycolatopsis SURUFUREA (Amycolatopsis sulphurea) (reference 1 same as the above; and reference 2, "International Journal of Systematic Bacteriology" 37 a volume, 292 - 295 pages, 1987) as a kind of a close relationship. Then, MK299-95F4 share and this laboratory preservation strain of Amycolatopsis SURUFUREA are [comparison] under examination to practice, this time — MK299-95F4 share — Amycolatopsis ESUP1 (Amycolatopsis sp.) — it is referred to as MK299-95F4. In addition, the deposition application of the MK299-95F4 share was made in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, and it was entrusted with the deposition number as FERM P-15243 on October 17, Heisei 7.

[0032] In enforcing the approach of the 2nd this invention, the production bacillus of the epoxy kino mycin C and D belonging to the Amycolatopsis group is inoculated into a nutrition culture medium, and it cultivates in this culture medium. The nutrition culture medium used here contains the carbon source and nitrogen source which can carry out utilization of the aforementioned production bacillus as a nutrition component.

[0033] As the nutrient, nutrients which can be assimilated, such as what is usually used as a nutrient of a microorganism, for example, a carbon source, a nitrogen source, and mineral salt, can be used. For example, the mineral salt of dipotassium phosphate, sodium phosphate, salt, a calcium carbonate, magnesium sulfate, a manganese chloride, etc. can be used for nitrogen sources, such as the carbon source like fats and oils, such as carbohydrates, such as grape sugar, a maltose, molasses, a dextrin, a glycerol, and starch, and soybean oil, peanut oil, and a peptone, a meat extract, cottonseed powder, a soybean meal, a yeast extract, casein, corn steep liquor, N2-amine, an ammonium sulfate, an ammonium nitrate, and an ammonium chloride and a pan, and a trace element, for example, cobalt, iron etc. be added as occasion demands. If a use bacillus can use for producing antibiotic epoxy kino mycin C and D in addition to this as a nutrient, any well-known nutrient can be used.

[0034] Especially the blending ratio of coal of the nutrient like the above in a culture medium is not restrained, can continue broadly and can be changed, and if the optimal presentation and the optimal blending ratio of coal of a nutrient are a person concerned by the epoxy kino mycin C to be used and D production bacillus, an easy bench scale test can determine easily. Moreover, as for the nutrition culture medium which consists of the above-mentioned nutrient, it is advantageous to be able to sterilize in advance of culture and to adjust pH of a culture medium in front of this sterilization or in the back in the range of 6-8, especially the range of pH 6.5-7.5.

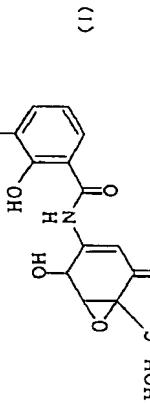
[0035] Culture of the epoxy kino mycin C in this nutrition culture medium and D production bacillus can be performed according to the approach usually used in manufacture of the antibiotic by the common Actinomycetes. Usually, it can carry [while cultivating under an aerobic condition is suitable and it stirs, and/or] out, carrying out aeration. Moreover, although both stationary culture shaking culture and the submerged culture accompanied by aeration stirring are usable as the culture approach, liquid culture is suitable for mass production method of

epoxy kino mycin C and D.

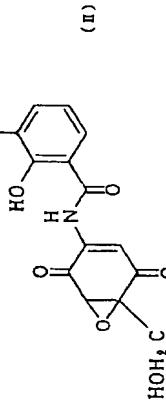
[0036] Although the culture temperature which can be used can be suitably chosen according to the production bacillus which growth of epoxy kino mycin C and D production bacillus is not substantially checked, it is not especially restricted if it is the range which can produce this antibiotic, and is used, especially a desirable thing can mention the temperature within the limits of 25 to 30 degree C. Culture is continual until epoxy kino mycin C and D is usually fully accumulated. Although the culture time amount changes with the presentation of a culture medium, culture temperature and service temperature, use production strain, etc., the target antibiotic can usually be obtained by culture of 72-120 hours. The accumulated dose of the epoxy kino mycin C and D in the culture medium under culture can use staphylococcus AUREUSU Smith, and he can do a quantum with the cup method used for the quantum of the usual antibiotic.

[0037] The epoxy kino mycin C and D accumulated into the culture in this way extracts this from a culture. After culture, as occasion demands, after removing a fungus body from a culture by the separation approaches well-known in itself, such as filtration and centrifugal separation. The solvent extraction adjust the culture filtrate to acidity (pH 2-4), and using an organic solvent, especially ethyl acetate, etc.. Isolation purification of the chromatography using the chromatography and gel filtration using adsorption or ion-exchange ability, and countercurrent distribution can be carried out by using it, being independent or combining, and the target antibiotic can be extracted. As support for chromatographies which has adsorption and ion-exchange ability, activated carbon, silica gel, porous polystyrene-divinylbenzene resin, or various kinds of ion exchange resin can be used. Moreover, from the separated fungus body, the target antibiotic can be extracted from a fungus body by the solvent extraction method using a suitable organic solvent, or the melting by fungus body crushing, and isolation purification can be carried out like the above. The new molecular entity epoxy kino mycin C and D which has the above mentioned property in this way is obtained.

[0038] Furthermore, the general formula aforementioned in the 3rd this invention (I)



They are the antibiotic epoxy kino mycin C which is expressed with (R showing a hydrogen atom by epoxy kino mycin C, and showing a chlorine atom by epoxy kino mycin D among a formula) and which is a compound and which is a compound, epoxy kino mycin D, and the following general formula (II).



The antirheumatic characterized by containing at least one compound chosen from the antibiotic epoxy quinomycin A which is expressed with (R showing a chlorine atom by epoxy quinomycin A, and showing a hydrogen atom by epoxy kino mycin B among a formula), and which is a compound and epoxy kino mycin B, or these salts as an active principle is offered. [0039] In the antirheumatic by the 3rd this invention, the epoxy kino mycin or the pharmaceutically permissible salt of those as an active principle can be a formal constituent with which it is mixed with the solid-state of pharmaceutically permissible daily use or liquid support,

for example, ethanol, water, starch, etc.

[0040] The epoxy quinomycin A and the epoxy kino mycin B which are used as an active principle with the antiarthematic by the 3rd this invention are the new matter, and the detail of these physicochemical qualities is Japanese Patent Application No. It is indicated by 7 No. - 3155/2 specification, and is Amycolatopsis of the above [specification / this]. Those manufacturing methods by culture of sp. MK 259-95F4 share are also indicated.

[0041] The main places of epoxy quinomycin A and the physicochemical quality of B are indicated below.

(1) epoxy quinomycin A is physicochemical — description — A appearance and property: — light yellow fine particles and weak acidic matter B melting point: 168 to 173 degree C (decomposition)

C) 25+44.6 degrees (c 0.51, methanol) of specific-rotation:[alpha] D

D) The Rf value of TLC: when it developed and measures with a chloroform-methanol (10:1) as an expansion solvent with the thin-layer chromatography of 0.28 silica gel (Art.105715, Merck Co. make).

[0042] E) molecular formula: — C₁₄H₁₀NO₆ ClF ultraviolet absorption spectrum: — the main peaks of UV absorption spectrum measured in the methanol solution are as follows, lambdamax nm (epsilon)₂₃₆ (sh.8900), 255 (sh.5900), 325 (8000), a 370(sh. 2700) G infrared absorption spectrum (KBr briquette method)

numax (cm⁻¹) 3450, 1710, 1610, 1600, 1520, 1460, 1340, 1230 [0043] (2) epoxy kino mycin B is physicochemical — description — A appearance and property: — light yellow fine particles and weak acidic matter B melting point: 178 to 184 degree C (decomposition)

C) The Rf value of 25+32.2 degree (c 0.51, methanol) DTLC of specific-rotation:[alpha] D : when it developed and measures with a chloroform-methanol (10:1) as an expansion solvent with the thin-layer chromatography of 0.52 silica gel (Art.105715, Merck Co. make).

[0044] E) molecular formula: — C₁₄H₁₁NO₆F ultraviolet absorption spectrum: — the main peaks of UV absorption spectrum measured in the methanol solution are as follows, lambdamax nm (epsilon)₂₃₇ (6100), 253 (sh. 5400), a 326(6300) G infrared absorption spectrum (KBr briquette method)

numax (cm⁻¹) 3430, 1710, 1660, 1610, 1530, 1340, 1230 [0045] The epoxy kino mycin C and D, the epoxy quinomycin A, and B which are used as an active principle with the antiarthematic by the 3rd this invention have the activity which controls the collagen induction arthritis which is the animal experiment model of arthritis-chronica rheumatism as aforementioned. When epoxy kino mycin C and D, epoxy quinomycin A, and especially B are used as an antiarthematic, although those doses change with symptoms, generally, the adult daily dose of 10-2000mg, they are 20 to 600 mg preferably, and it is good [doses] to prescribe a medicine for the patient in 1 - 3 steps as occasion demands according to a symptom. A medication method can take the gestalt suitable for administration, and is especially desirable. [of oral administration or vein-administration]

[0046] Since epoxy quinomycin A - D have the depressant action to collagen induction arthritis as they are shown above, they can expect not only rheumatoid arthritis but to apply as autoimmune mitigation or an inhibitor effective also in prevention or the therapies of an autoimmune disease, such as systemic lupus erythematosus, systemic sclerosis, a periarthritis nodosa, ulcerative colitis, and juvenile diabetes.

[0047] [Embodiment of the Invention] Next, although an example explains this invention to a detail further, this invention is not limited to the following example.

[0048] Example 1 Antibiotic epoxy kino mycin C and D, epoxy quinomycin A, and manufacture (A) glycerol of B 0.5%, shoe cloth 2%, soybean meal 1%, 1% of dry yeast, corn steep liquor 0.5%, cobalt chloride Liquid medium containing 0.001% (it adjusts to pH7.0) Erlenmeyer flask (500ml ***) it pours 110ml distributively at a time, and is a conventional method. It sterilized at 120 degrees C for 20 minutes. Amycolatopsis sp. cultivated to these culture media at the agar slant medium MK259-95F4. The stock (FERM P-15243) was inoculated and rotary shaking culture was carried out for five days at 30 degrees C after that. This obtained **** culture medium.

[Translation done.]

[0049] Glycerol 2%, dextrin 2%, bacto-SOITON 1%, powder yeast extract 0.3%, ammonium sulfate 0.2%, calcium carbonate Liquid medium which contains one drop of silicone oil 0.2% (it adjusts to pH7.4) Erlenmeyer flask (500ml ***) It pours 110ml distributively at a time, and is a conventional method. It sterilized at 120 degrees C for 20 minutes. Then, it inoculated the 2ml of the above-mentioned **** culture medium into these culture media at a time, respectively, and rotary shaking culture was carried out to them for 27 degrees C.

[0050] Thus, centrifugal separation of the obtained culture medium was carried out, and the fungus body was removed. Culture filtrate 1.8L (L) is 6 N-HCl. It is butyl acetate after making it pH2. It extracted by 1.8L, and the butyl-acetate layer was dried with anhydrous sodium sulfate. Concentration hardening by drying was carried out under reduced pressure of a butyl-acetate layer, residue was melted to methanol 50ml, and it washed twice by hexane 50ml. When concentration hardening by drying of the methanol layer was carried out under reduced pressure, brown oily matter (980mg) was obtained. If this oily matter is given to a silica gel column (Merck, Kieselgel 60, 120ml) and sequential elution is carried out by the toluene-acetone system (1:five: 10:1, 3:1), the mixture of 19mg and epoxy kino mycin C and D 170mg was obtained. [epoxy quinomycin A] [18mg and epoxy kino mycin B] When separation purification of 5 mg of this mixture was carried out with silica gel TLC (Merck, Art.105715, a chloroform-10% water methanol = it develops 3 times by 10:1) 13mg of epoxy kino mycin C of a white solid-state was obtained, and 23mg of epoxy kino mycin D of yellowish brown powder was obtained. That is, epoxy kino mycin C is the melting point. It is obtained with the yield of 13mg as 168-172 degrees C (decomposition) white powder, and epoxy kino mycin D is the melting point. It was obtained with the yield of 23mg as yellowish brown powder of 163 to 168 degree C (decomposition).

(B) The culture medium obtained like the still more nearly aforementioned (A) term was filtered, and the fungus body was separated. In 2.55L (L) of culture filtrates, it is 6 N-HCl. After making it pH2, it extracted by butyl-acetate 2.55L, and the butyl-acetate layer was dried with anhydrous sodium sulfate. Concentration hardening by drying was carried out under reduced pressure of a butyl-acetate layer, residue was melted to methanol 50ml, it washed twice by hexane 50ml, and concentration hardening by drying was carried out under reduced pressure of a methanol layer. It is chloroform-methanol-water (50:10:40,100ml) about the obtained residue. If it distributes and brown oily matter (0.515g) It was obtained. This oily matter was given to the silica gel column chromatography (Kieselgel 60, the Merck Co. make, 50ml), and sequential elution was carried out with the toluene-acetone mixed solvent (1 three: 10:1, 7:1, 5:1, 2:1). The obtained activity fraction was given to the silica gel column chromatography of these conditions, and sequential elution was carried out with the toluene-acetone mixed solvent (1 ten: 50:1, 20:1, 7:1). Epoxy quinomycin A and the mixture of B 124mg was obtained. Separation purification was carried out having bet 35mg of this mixture on silica gel TLC (expansion solvent: a chloroform-methanol, 20:1). Epoxy quinomycin A is the melting point. It is obtained with the yield of 20mg as light yellow powder of 168 to 173 degree C (decomposition) and epoxy kino mycin B is the melting point. It was obtained with the yield of 10mg as light yellow powder of 178 to 184 degree C (decomposition).

* NOTICES *

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- 1.This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.**** shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] The inside of the methanol solution of epoxy kino mycin C. 0.01Ns It is each ultraviolet absorption spectrum in a NaOH-methanol solution and a 0.01N HCl-methanol solution.

[Drawing 2] It is the infrared absorption spectrum measured with the KBr briquette method of epoxy kino mycin C.

[Drawing 3] It is the proton nuclear-magnetic-resonance spectrum measured with the heavy methanol solution (internal standard: trimethyl silane) of epoxy kino mycin C.

[Drawing 4] It is the carbon 13 nuclear-magnetic-resonance spectrum measured with the heavy methanol solution (internal standard: trimethyl silane) of epoxy kino mycin C.

[Drawing 5] The inside of the methanol solution of epoxy kino mycin D. 0.01Ns It is each ultraviolet absorption spectrum in a NaOH-methanol solution and a 0.01N HCl-methanol solution.

[Drawing 6] It is the infrared absorption spectrum measured with the KBr briquette method of epoxy kino mycin D.

[Drawing 7] It is the proton nuclear-magnetic-resonance spectrum measured with the heavy methanol solution (internal standard: trimethyl silane) of epoxy kino mycin D.

[Drawing 8] It is the carbon 13 nuclear-magnetic-resonance spectrum measured with the heavy methanol solution (internal standard: trimethyl silane) of epoxy kino mycin D.

[Translation done.]